1315630

PATENT SPECIFICATION

(11) 1 315 630

NO DRAWINGS

(21) Application No. 8754/71 (22) Filed 31 March 1971

(23) Complete Specification filed 21 March 1972

(44) Complete Specification published 2 May 1973

(51) International Classification C07D 99/14; A61K 21/00

(52) Index at acceptance

C2A 1C1A 1C1B 1C2A 1C2B 1C2C 2C1

(72) Inventors GEORGE ROBERT FOSKER and WILLIAM DAVIES

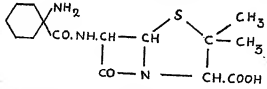
(19)

(54) PENICILLINS

(71) We, BEECHAM GROUP LIMITED, a British Company, of Beecham House, Great West Road, Brentford, Middlesex, England, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to new penicillins and is particularly concerned with a new class of penicillins which are derivatives of 6-aminopenicillanic acid and which are of value as antibacterial agents, as nutritional supplements in animal foods, as agents for the treatment of mastitis in cattle and as therapeutic agents in poultry and animals, including man, in the treatment especially of infectious diseases caused by Gram-positive and Gram-negative bacteria.

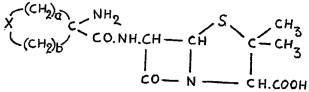
The penicillin of formula (I) is a known compound which has a relatively low order of activity *in vitro* against both Gram-positive and Gram-negative organisms.



(I)

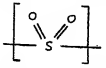
However, the penicillin (I) is extremely well absorbed *in vivo* and as a result, it is believed to be therapeutically equivalent to the most widely used broad-spectrum penicillin, α-aminobenzylpenicillin.

According to the present invention there is provided a class of penicillins having the general formula (II)



(II)

and salts and esters thereof, in which formula X represents -[-O-]-, -[-S-]-,



or [R—N]< wherein R represents hydrogen, alkyl or aralkyl and a and b are each integers such that a+b=4.

The salts are non-toxic salts including non-toxic metallic salts such as sodium, potassium, calcium and aluminium, ammonium and substituted ammonium salts, e.g. salts of such non-toxic amines as trialkylamines, including triethylamine, procaine,

]

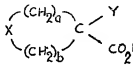
dibenzylamine, N-benzyl-beta-phenethylamine, 1-ephenamine, N,N'-dibenzylethylenediamine, dehydroabietylamine, N,N'-bis-dehydroabietylethylenediamine, and other amines which have been used to form salts with benzylpenicillin. Acid addition salts of the compounds of formula (II) are also included within the scope of the present invention.

The esters of the penicillins of this invention are non-toxic esters, particularly those which are easily de-esterified in the body to give the parent penicillanic acid derivatives. Examples of such esters include acyloxyalkyl esters particularly the acyloxymethyl esters such as the acetoxymethyl and pivaloyloxymethyl esters. It will be understood, of course, that the invention includes other esters which may be of value as intermediates en route to the penicillanic acid, e.g. silyl esters.

In a preferred class of compounds of this invention the integers a and b are such that a+b=4 e.g. when a=1, b=3 and when a=2, b=2.

On the basis of preliminary testing, many of the compounds of this invention have a remarkably higher level of *in vitro* antibacterial activity against Gram-negative organisms than the known compound of formula (I), and yet are very well absorbed, giving them therapeutic advantages over compound (I).

The penicillins, penicillin salts and penicillin esters of this invention may be prepared by a process wherein 6-aminopenicillanic acid or a salt or ester thereof, or silyl 6-aminopenicillanic acid is treated with a reactive N-acylating derivative of an acid of formula (III)



(III)

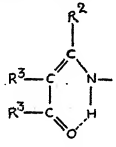
wherein X, a and b are as defined in formula (II) and Y is a nitrogen-containing group which may be converted into a primary amino group, and subsequently converting the group Y into a primary amino group.

The conversion of the nitrogen-containing group Y of the intermediate penicillin to a primary amino group may be effected by hydrogenation or hydrolysis it being understood that both steps must be carried out under conditions sufficiently mild that they do not disrupt the sensitive β-lactam ring. When the amino group is protected by protonation only, conversion of Y to NH_2 merely requires adjustment of pH.

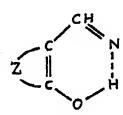
The reactive derivative of the acid (III) may be the acid halide, azide anhydride, mixed anhydride, or the reactive intermediate formed from the acid and a carbodiimide or carbonyldiimidazole.

Examples of the nitrogen-containing group Y which, in the intermediate penicillin can be converted into the primary amino group by a process of catalytic hydrogenation include the azido group, the benzyloxycarbonylamino group, and substituted benzyloxycarbonylamino group.

Examples of the group Y which may be converted into a primary amino group by a process of mild acid hydrolysis include enamine groups of general formula (IV), or tautomeric modifications thereof and *o*-hydroxyarylideamino groups of the general formula (V), or tautomeric modifications thereof. More detailed descriptions of the use of these groups in the synthesis of amino-penicillins appear in British Patent Specifications 991,586 and 980,777:—



(IV)



(V)

In structures (IV) and (V) the dotted lines represent hydrogen bonds. In structure (IV) R^1 is a C_1 to C_6 alkyl group, R^2 is either a hydrogen atom or together with R^1 completes a carbocyclic ring, and R^3 is a C_1 to C_6 alkyl, aryl or C_1 to C_6 alkoxy group. In structure (V) Z represents the residue of a substituted or unsubstituted benzene or naphthalene ring.

Another example of a group Y which can be converted into H_2N after coupling of the acid (III) with 6-amino-penicillanic acid is the azido group. In this case the final conversion into NH_2 may be brought about either by catalytic hydrogenation or by electrolytic reduction.

In carrying out the coupling of the acid (III) to 6-aminopenicillanic acid the choice of activating group for the carboxyl function will be influenced by the chemical nature of the α-substituent Y. Thus, when Y is an acid-stable group, such as the protonated amino group NH_3^+ or the azido group, it is often convenient to convert the acid (III) into an acid halide, for example by treating it with thionyl chloride or phosphorous pentachloride to give the acid chloride. Such reagents would however be avoided when X is an acid labile group or type (IV) or (V), in which case it is often convenient to make use of a mixed anhydride. For this purpose particularly convenient mixed anhydrides are the alkoxyformic anhydrides, which are conveniently prepared by treating an alkali metal or tertiary amine salt of the acid (III) with the appropriate alkyl chloroformate in an anhydrous medium at or below room temperature. Other ways of activating the carboxyl group include reaction with a carbodiimide to give a reactive O-acyl isourea or reaction with carbonyldiimidazole to give a reactive imidazolide. These latter derivatives, like the mixed anhydrides, are relatively unstable substances and hence are not usually isolated, the reaction with 6-aminopenicillanic acid being carried out *in situ*.

Another reactive N-acylating derivative of the acid (III) useful in the preparation of the compound of the present invention is the Leuch's anhydride. In this structure the groups which activates the carboxyl group also serves to protect the amino group.

The compounds of the present invention may be isolated in any of the ways customarily employed for the isolation of aminopenicillins. Thus it may be obtained as the neutral molecule, although this is probably more accurately represented as the zwitterion, or it may be isolated as a salt. Since the molecule contains both basic and acidic functions the salts are of two kinds. The acid addition salts, some of which are sparingly soluble in water and thus useful for isolation purposes, include salts with mineral acids such as hydrochloric, phosphoric, or thiocyanic acid as well as with strong organic acids such as naphthalene-β-sulphonic acid. The base addition salts include alkali and alkaline earth metal salts, the ammonium salt, and salts with non-toxic amines. Any of these forms may be either anhydrous or hydrated. They may also be either amiorphous or crystalline, but the crystalline forms are preferred since they have greater stability.

At any suitable stage in the process the material may be subjected to purification procedures designed to remove traces of high molecular weight allergenic impurities.

The penicillins of the present invention may be employed in admixture with suitable pharmaceutical carriers in various medicinal dosage forms. Additionally, the penicillins may be employed in synergistic combinations with known penicillinase-resistant penicillins, for example, methicillin, cloxacillin, dicloxacillin, flucloxacillin and nafcillin.

The novel penicillins, salts and esters of this invention where a and b are not simultaneously equal to 2 are capable of existing in two epimeric forms and it is to be understood that this invention includes both the D- and L- forms as well as the DL-mixture of such compounds.

Certain embodiments of the invention will now be illustrated by the following specific Examples:—

Example 1

Preparation of 6-[4-amino-1-thiacyclohexan-4-yl carbamido] penicillanic acid 1-thiacyclohexan-4-one, [(13.5 g.), prepared as described in the literature by Johnson and Berchtold, J. Org. Chem., 1970, *35*, 587], dissolved in ethyl alcohol, (150 ml.), was added to a stirred suspension of sodium cyanide, (8.54 g.), and ammonium carbonate, (40.6 g.), in water, (150 ml.). The mixture was heated at 65° for 3 hours, a clear solution was obtained after 1 hour. The bulk of the solution was reduced by one third by evaporation under reduced pressure, where upon cooling the concentrate to 5° white crystals were obtained. The crystals were collected by filtration and dried *in vacuo* over phosphorus pentoxide to give 1-thiacyclohexan-4-yl-5'-spiro hydantoin, [(9.07 g.), m.p. 256° (d), found C, 45.2; H, 5.4; N, 14.8; S, 17.3;

$C_{14}H_{18}N_2O_2S$ requires C, 45.1; H, 5.4; N, 15.0: S, 17.2%. i.r.γ max. (mull) 1770 cm^{-1}, 1735 cm hydantoin carbonyls].

The hydantoin, (9.0 g.), barium hydroxide-$8H_2O$, (31.5 g.), and water, (160 ml.), were heated in a sealed autoclave at 160° for 2 hours, cooled and filtered. Ammonium carbonate, (8 g.), was added to the filtrate, which was re-filtered and the filtrate evaporated to dryness under reduced pressure. The resultant white solid was slurried with cold water, (10 ml.), and filtered. The crystalline product was air dried at 100° for 2 hours to give 4-amino-1-thiacyclohexan-4-yl carboxylic acid, [(3.28 g.), m.p. 270° (d), found C, 44.5; H, 6.9; N, 8.6; S, 19.6; $C_6H_{11}NO_2S$ requires C, 44.7; H, 6.9; N, 8.7; S, 19.9%, i.r.γ max. (mull) 1615 cm^{-1} carboxylate CO].

The amino acid, (2.31 g.), was suspended in dry methylene dichloride, (50 ml.), and dry hydrogen chloride gas passed for one hour. The suspension was cooled in an ice-salt bath, phosphorus pentachloride, (9.5 g.), was added portionwise over 10 minutes and then stirred for 2 days further with the exclusion of moisture. The white solid was quickly, but carefully, collected by filtration, dried *in vacuo* over phosphorus pentoxide to give 4-amino-1-thiacyclohexan-4-yl carbonyl chloride hydrochloride, [(1.0 g.), found Cl, 32.3; $C_6H_{11}Cl_2NOS$ requires Cl, 32.8%. i.r.γ max. (mull) 1770 cm^{-1} acid chloride carbonyl].

Triethylamine, (1.61 ml.), and N,N-dimethylaniline, (0.66 ml.), were added to 6-aminopenicillanic acid, (0.99 g.), suspended in dry methylene dichloride, (10 ml.) and heated at reflux for 1 hour. After cooling to 12—15° under dry nitrogen, trimethylchlorosilane, (1 g.), was added dropwise to the mixture, which, was then heated at reflux for a further 45 minutes and finally chilled to −15° with the continuous passage of dry nitrogen. 4-amino-1-thiacyclohexan-4-yl carbonyl chloride hydrochloride (1.0 g.), was added portionwise with continuous stirring. Stirring was continued for 30 minutes further, then the reaction mixture poured into cold water, (20 ml.) and filtered through kieselguhr. The pH of the two phases was adjusted to 5.4 with dilute sodium hydroxide solution and the phases separated. The aqueous phase was further extracted with methylene dichloride, (2 × 20 ml.), separated, concentrated to half volume under reduced pressure and temperature and then kept at 5° overnight. The crystalline precipitate was collected by filtration, air dried at 40° for 3 hours to give 6-[4-amino-1-thiacyclohexan-4-yl carbamido]-penicillanic acid, [(1.0 g.), i.r. γ max (mull) 1775 cm^{-1} β lactam, 1690 cm^{-1} amide carbonyl]. This material when subjected to paper chromatography in butanol: ethanol: water revealed a single zone of antibacterial inhibition at an R_f value of 0.23 and was estimated by colorimetric assay with hydroxylamine to be 79% pure.

Example 2

Preparation of 6-[4-amino tetrahydropyran-4-yl carbamido] penicillanic acid

Cheldonic acid (76 g.) was thermally decarboxylated and re-distilled to give pyran-4-one (19.5 g.) which was then hydrogenated in methanol (200 ml.) at atmospheric pressure for one hour using 5% palladium/calcium carbonate (9.0 g.) as catalyst. After removal of the catalyst and the solvent, the concentrate was distilled to yield tetrahydro pyran-4-one [(14.0 g.) b.p. 162—4°].

This ketone (12.3 g.) in ethanol (100 ml.) was added dropwise to a stirred suspension of sodium cyanide (9.1 g.) and ammonium carbonate (58.2 g.) in water (100 ml.). The mixture was heated at 65° for 4 hours, becoming clear after about 2 hours. It was then reduced to half-volume by distillation, the concentrate chilled to 0° and the resultant white crystalline precipitate removed by filtration. The filtrate was acidified with concentrated hydrochloric acid and the resultant precipitate collected by filtration. The combined solids (10.6 g.) were recrystallised from ethanol/petroleum ether 40—60° to give, after drying *in vacuo* over phosphorus pentoxide, tetrahydro pyran-4-yl-5'-spirohydantoin [(7.1 g.) m.p. 262—3° (d), found C, 49.1; H, 5.9; N, 16.3; $C_8H_{12}N_2O_3$ requires C, 49.4; H, 5.9; N, 16.4%, i.r. γ max (nujol) 1770 and 1735 cm^{-1} hydantoin CO's].

The hydantoin (5.1 g.), lithium hydroxide (6.3 g.) were dissolved in water (100 ml.) and the clear solution gently heated under reflux until a sample of the reaction mixture, when subjected to thin layer chromatography, indicated only the presence of the amino acid, (approximately 44 hours). The mixture was cooled to room temperature, filtered, and the filtrate concentrated under reduced pressure. The pH of the concentrate was adjusted from 12.5 to 4.9 with concentrated hydrochloric acid and then evaporated to dryness under reduced pressure. Methanol (35 ml.) was added to the residue, the white precipitate removed by filtration, thoroughly washed with methanol and dried *in vacuo* over phosphorus pentoxide to give the crude amino acid (4.3 g.)

The crude acid was recrystallised from aqueous ethanol to give 4-amino tetrahydropyran-4-yl carboxylic acid [3.7 g.), m.p. 282° (d), found C, 49.7; H, 7.7; N, 9.6; $C_6H_{11}NO_3$ requires C, 49.6; H, 7.6; N, 9.7%, i.r. γ max (nujol) 1615 cm^{-1} carboxylate CO, 2505 cm^{-1} NH_3^+].

The amino acid (1.5 g.), was suspended in dry methylene dichloride (20 ml.), chilled to 0 to 5° in an ice-bath and dry hydrogen chloride gas passed into the mixture at this temperature for 90 minutes with the exclusion of moisture. Phosphorus pentachloride (6.2 g.) was added in one portion and then vigorously stirred at 0 to 5° for 5 hours with the further passage of dry hydrogen chloride gas and for 16 hours with no further external cooling or hydrogen chloride gas.

Acetone (4 ml.) and dry diethyl ether were cautiously added, the resultant suspension filtered in a dry-box, well washed with dry diethyl ether and dried *in vacuo* over phosphorus pentoxide to give 4-amino tetrahydropyran-4-yl carbonyl chloride hydrochloride [(1.98 g.) i.r. γ max (nujol) 1765 cm^{-1} acid chloride CO]. As the material was sensitive to moisture it was used immediately in the preparation of the penicillin.

Freshly preformed triethylammonium 6-aminopenicillanate (3.17 g.) was dissolved in dry methylene dichloride (20 ml.), triethylamine (2.1 ml.) and N,N-dimethyl aniline (1.52 g.) at room temperature under the continuous passage of dry nitrogen which was continued throughout the rest of the preparation. The clear solution was cooled to 5° and trimethylchlorosilane (2.56 ml.) added dropwise with stirring. The temperature rose to 13° with the formation of a thick white precipitate which was gently heated on a water-bath under reflux for 45 minutes. It was then cooled to − 30° and maintained at this temperature whilst the acid chloride hydrochloride (1.92 g.) was added portionwise over 2 to 3 minutes with vigorous stirring. This was continued for 30 minutes further at − 15° and then poured into ice-cold water (40 ml.). The two phase system was filtered through a Kieselguhr pad and adjusted from pH 2.1 to 5.4 with triethylamine. The aqueous layer was separated, washed twice further with methylene dichloride (2 × 50 ml.), then evaporated to dryness under reduced pressure and dried *in vacuo* over phosphorus pentoxide. The resulting crude penicillin (7.5 g.) was thoroughly triturated with dry methylene dichloride (200 ml.), filtered and finally dried *in vacuo* over phosphorus pentoxide to give the required penicillin [(2.3 g.) γ max (nujol) 1775 cm^{-1} β-lactam CO, 1680 cm^{-1} amide CO, [$(CD_3)_2SO$] 4.53 (2H, 2 doublets, C_5/C_6 protons), 5.27 (4H, complex multiplet, NH_3^+ and NH), 5.74 (1H, singlet, C_2 proton), 6.36 (4H, complex multiplet, C_2 and C_6 pyranyl), 8.04 (4H, complex multiplet, C_3 and C_5 pyranyl) 8.43 and 8.52 (6H, singlets, gem dimethyls). Addition of D_2O, the 5.27 peak disappears and the 6.36 peak now hidden by D_2O]. The product when subjected to paper chromatography in butanol : ethanol : water had an R_f value of 0.19 and was estimated to be 75% pure by colorimetric assay with hydroxylamine.

Example 3

Preparation of 6-[DL-3-amino-1-thiacyclohexan-3-yl carbamido] penicillanic acid

1-thiacyclohexan-3-yl-5'-spiro hydantoin was prepared essentially as described in Example 1 when 1-thiacyclohexan-4-one was replaced by 1-thiacyclohexan-3-one [(14.0 g.), prepared as described in the literature by Leonard and Figueros J. Amer. Chem. Soc., 1952, *74*, 9.71], and the hydantoin [(19.6 g.) m.p. 222°, found C, 44.5; H, 5.4; N, 15.0; $C_7H_{10}N_2O_2S$ requires C, 45.1; H, 5.4; N, 15.0%], was isolated after acidification of the chilled, reaction mixture concentrate.

The crude amino acid (10.8 g.) was isolated when this hydantoin (18.0 g.) was hydrolysed as described in Example 1 but on exactly twice the scale. The crude material was recrystallised from aqueous ethanol to give DL-3-amino-1-thiacyclohexan-3-yl carboxylic acid [(6.4 g.), m.p. 284° (d), found C, 44.6; H, 6.9; N, 8.5; $C_7H_{13}NO_2S$ requires C, 44.7; H, 6.9; N, 8.7% i.r. γ max (nujol) 1610 cm^{-1} carboxylate CO, 2600 cm^{-1} NH_3^+].

The amino acid (1.6 g.) was suspended in dry methylene dichloride (20 ml.), chilled in an ice-bath and treated with dry hydrogen chloride gas for 90 minutes with the exclusion of moisture. Phosphorus pentachloride (3.2 g.) was quickly added and the mixture worked up exactly as detailed in Example 2 to give DL-3-amino-1-thiacyclohexan-3-yl carbonyl chloride hydrochloride [(2.2 g.) i.r. γ max. (nujol) 1785 cm^{-1} acid chloride CO].

The penicillin [(2.2 g.), i.r. γ max (nujol) 1770 cm^{-1} β-lactam Co, 1670 cm^{-1} amide CO_2] was prepared and isolated exactly as described in Example 2 when 4-amino tetrahydropyran-4-yl carbonyl chloride hydrochloride was replaced with DL-3-amino-1-thiacyclohexan-3-yl carbonyl chloride hydrochloride (2.15 g.). This material

when subjected to paper chromatography in butanol:ethanol:water revealed a single zone of antibacterial inhibition at an R_f value of 0.23 and was estimated by colorimetric assay with hydroxylamine to be 66% pure.

Example 4

Preparation of 6-[4-amino-1,1-dioxo-1-thiacyclohexan-4-yl carbamido] penicillanic acid

An aqueous solution of potassium permanganate (3.16 g.) in water (75 mL) was added dropwise with vigorous stirring at room temperature to a clear solution of 4-amino-1-thiacyclohexan-4-yl carboxylic acid (4.0 g.) dissolved in 5N sulphuric acid (11 mL) and water (35 mL). After the addition was complete stirring was continued for 24 hours then the pH adjusted from 2.5 to 6.3 by the addition of triethylamine. The reaction mixture was passed down a 30 cm. Amberlite IRA 401 (Cl^-) column and then a 30 cm. Amberlite IRC 50 (Et_3NH^+) resin column when the final eluate (ca. 250 mL) was freeze dried (the word "Amberlite" being a Registered Trade Mark). The resulting solid was thoroughly triturated with methylene dichloride, filtered, and dried *in vacuo* over phosphorus pentoxide and then recrystallised from aqueous ethanol to give 4-amino-1,1-dioxo-1-thiacyclohexan-4-yl carboxylic acid, [(2.7 g.), m.p. 280° (d), found C, 37.6; H, 6.0; N, 7.2; S, 16.2 $C_7H_{11}NO_4S$ requires C, 37.3; H, 5.7; N, 7.3; S, 16.6%, i.r. γ max (nujol) 3150 cm^{-1} NH_2^+, 1585 cm^{-1} carboxylate CO, 1285 and 1110 cm^{-1} sulphone].

4-amino-1,1-dioxo-1-thiacyclohexan-4-yl carbonyl chloride hydrochloride, [(2.6 g.), i.r. γ max (nujol) 1770 cm^{-1} acid chloride CO, 1290 and 1115 cm^{-1} sulphone], was prepared when the amino acid (2.5 g.) was substituted for 4-amino tetrahydropyran-4-yl carboxylic acid exactly as described in Example 2.

The penicillin [(0.76 g.) i.r. γ max (nujol) 1770 cm^{-1} β-lactam CO, 1680 cm^{-1} amide CO, 1310 and 1120 cm^{-1} sulphone] was prepared and isolated exactly as in Example 2 when the above acid chloride hydrochloride (2.5 g.) was substituted for the acid chloride hydrochloride used in that example. The product when subjected to paper chromatography in butanol:ethanol:water revealed an R_f value of 0.11 and was estimated to be 47% pure by colorimetric assay with hydroxylamine.

Example 5

Preparation of 6-[4-amino-1-benzylpiperid-4-yl carbamido] penicillanic acid

1-benzyl-4-yl-5'-spiro hydantoin [(27.8 g.) m.p. 256° (d) i.r. γ max (nujol) 1730 and 1770 cm^{-1} hydantoin CO's] was isolated exactly as described in Example 2 when tetrahydropyran-4-one was replaced by N-benzylpiperid-4-one (26.7 g.)

Similarly 4-amino-1-benzylpiperidin-4-yl carboxylic acid, [(11.4 g.), m.p. 247° (d), i.r. γ max (nujol) 1615 cm^{-1} carboxylate CO, found C, 66.7; H, 7.7; N, 11.9; $C_{13}H_{18}N_2O_2$ requires C, 66.7; H, 7.7; N, 12.0%], was obtained, then the above hydantoin (24.0 g.) was substituted for the hydantoin described in Example 2, but on three times the scale.

Phosgene was passed slowly through a stirred suspension of the amino acid (4.7 g.) in dry dioxan (50 mL) at 40° for 4 hours with the careful exclusion of moisture. The mixture was stirred for a further 15 hours at room temperature and then evaporated at low temperature and pressure to a gummy solid. The crude material was refluxed with dry tetrahydrofuran (300 mL), cooled and diluted with dry 40—60° petroleum ether (250 mL) to give the corresponding Leuch's anhydride hydrochloride, [(5.7 g.) m.p. *ca* 94° (d), i.r. γ max (nujol) 2350, 2400, 2550 and 2650 cm^{-1} NH^+, 1720 cm^{-1} CO, 1780 and 1850 cm^{-1} '5' ring anhydride].

A suspension of 6-amino penicillanic acid (1.9 g.) in water (7.0 mL), tetrahydrofuran (18 mL) with stirring was adjusted to pH 7 with triethylamine and cooled to 0°. The solution was treated portionwise over 20 minutes with the Leuch's anhydride hydrochloride (2.6 g.) with the simultaneous addition of triethylamine to maintain the pH at 6.5. The mixture was stirred further for 1 hour at 0°, diluted with an equal volume of water and evaporated to dryness at low temperature and pressure. The residue was dissolved in methylene dichloride, filtered, and again evaporated to dryness to give the crude yellow-white penicillin (6.7 g.). The crude product was treated with *n*-butanol (10 mL) and filtered. Dry ether (100 mL) was added to the filtrate, which after collection, drying *in vacuo* yielded a yellow glass (2.15 g.). This material upon trituration with absolute ethanol (25 mL) gave the required penicillin, [(0.82 g.) i.r. γ max (nujol) 1670 cm^{-1} amide CO, 1775 cm^{-1} β-lactam CO,], as a white stable solid. The penicillin, when subjected to paper chromatography in butanol:ethanol:water indicated an R_f value of 0.46 and when subjected to colorimetric assay with hydroxylamine was shown to be 67% pure.

Example 6

Preparation of 6-[4-aminopiperidin-4-yl carbamido] penicillanic acid

A solution of 6-[4-amino-1-benzylpiperidin-4-yl carbamido] penicillanic acid (0.43 g.) in water (20 ml.) was hydrogenated at atmospheric pressure over prehydrogenated 30% palladium on barium carbonate (0.9 g.) for 5½ hours. The reaction mixture was filtered and the filtrate evaporated at low temperature and pressure to give the required penicillin, [(0.07 g.), i.r. γ max (nujol) 1765 cm^{-1} β-lactam CO.]. This material, when subjected to paper chromatography in butanol: ethanol: water revealed a single zone of R, 0.06 and was estimated to be 21% pure by colorimetric assay with hydroxylamine.

Example 7

By reacting the triethylamine salts of the penicillins of Examples 1 to 6, with bromomethylpivalate, or bromomethylacetate in an inert solvent, the corresponding pivaloyloxymethyl- and acetoxymethyl- esters of the penicillins are prepared.

Example 8

The following experiment shows that the concentration of 6-[4-aminotetrahydropyran-4-yl carbamido] penicillanic acid found in the serum of squirrel monkeys dosed by mouth with the compound were greater than the concentration of the known compound cyclacillin (I) achieved when that compound was administered in the same way. Each compound was given orally as a suspension to fasting monkeys at a dose of 100 mg/kg. Serum samples were taken at limited intervals and assayed for the penicillin.

TABLE

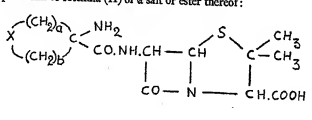
Compound administered	Concentration in ug/ml of penicillin in serum				
	hr	1 hr	2 hrs	4 hrs	6 hrs
Cyclacillin	58.5	46.2	19.1	4.5	1.0
6-[4-amino-tetrahydropyran-4-yl carbamido] penicillanic acid	62.7	75.4	29.7	3.3	0.4

Although the compounds of Examples 1, and 3 to 5 have not been subjected to rigorous absorption experiments, preliminary results indicate that they are well absorbed when given by the oral route.

Also, preliminary *in vitro* antibacterial tests have shown that the compounds of Examples 1 to 4 and 6 are as active as cyclacillin (I) against many organisms and more active than cyclacillin against some of those organisms most commonly encountered in clinical practice. The compounds of Example 1 and 2 are, broadly speaking, from 5 to 10 times more active than cyclacillin, according to our preliminary results. The compound of Example 5 appears to be generally slightly less active than cyclacillin against most of our test organisms.

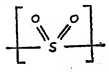
WHAT WE CLAIM IS:—

1. A penicillin of formula (II) or a salt or ester thereof:



(II)

wherein X represents -[-O-]-, -[-S-]-,



or [R—N]< wherein R is hydrogen, alkyl or aralkyl and a and b are each integers such that a+b=4.

2. A penicillin, penicillin salt or penicillin ester as claimed in claim 1 wherein the integer a in formula (II) is 2 and the integer b in formula (II) is 2.

3. A penicillin, penicillin salt or penicillin ester as claimed in claim 1 wherein the integer a in formula (II) is 1 and the integer b in formula (II) is 3.

4. 6-[4-amino-1-thiacyclohexan-4-yl carbamido] penicillanic acid and salts and esters thereof.

5. 6-[4-aminotetrahydropyran-4-yl carbamido] penicillanic acid and salts and esters thereof.

6. 6-[3-amino-1-thiacyclohexan-3-yl carbamido] penicillanic acid and salts and esters thereof.

7. 6-[4-amino-1,1-dioxo-1-thiacyclohexan-4-yl carbamido] penicillanic acid and salts and esters thereof.

8. 6-[4-amino-1-benzylpiperid-4-yl carbamido] penicillanic acid and salts and esters thereof.

9. 6-[4-aminopiperidin-4-yl carbamido] penicillanic acid and salts and esters thereof.

10. A penicillin, penicillin salt or penicillin ester as claimed in claim 1 and wherein a and b are not both equal to 2 said penicillin, penicillin salt or penicillin ester being in the form of a substantially pure optical isomer.

11. The hydrates of a penicillin, penicillin salt or penicillin ester as claimed in any one of claims 6 to 16.

R. SMITHER,
Chartered Patent Agent,
Agent for the Applicants.

Printed for Her Majesty's Stationery Office by the Courier Press, Leamington Spa, 1973.
Published by the Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.